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Comparison of Antimicrobial Properties of Silver Nanoparticles Synthesized from Selected Bacteria

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Abstract Green silver nanoparticle (AgNP) biosynthesis is facilitated by the enzyme mediated reduction of Ag ions by plants, fungi and bacteria. The antimicrobial activity of green AgNPs is useful to overcome the challenge of antimicrobial resistance. Antimicrobial properties of biosynthesized AgNPs depend on multiple factors including culture conditions and the microbial source. The antimicrobial activity of AgNPs biosynthesized by *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Acinetobacter baumannii* (confirmed clinical isolate) were investigated in this study. Biosynthesis conditions ($AgNO_3$ concentration, pH, incubation temperature and incubation time) were optimized to obtain the maximum AgNP yield. Presence of AgNPs was confirmed by observing a characteristic UV–Visible absorbance peak in 420–435 nm range. AgNP biosynthesis was optimal at 0.4 g/L $AgNO_3$ concentration under alkaline conditions at 60–70 °C. The biosynthesized AgNPs showed higher stability compared to chemogenized AgNPs in the presence of electrolytes. AgNPs synthesized by *P. aeruginosa* were the most stable while NPs of *S. aureus* were the least stable. AgNPs synthesized by *P. aeruginosa* and *S. aureus* showed good antimicrobial potential against *E. coli, P. aeruginosa, S. aureus,* MRSA and *Candida albicans*. AgNPs synthesized by *S. aureus* had greater antimicrobial activity. The antimicrobial activity of NPs may vary depending on the size and the morphology of NPs.

Abbreviations
AgNPs   Silver nanoparticles
ZOI   Zone of inhibition
CFU   Colony forming units

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Introduction

Green synthesis of nanoparticles (NPs) using bacteria has several advantages due to its inherent ecofriendly and economical nature. Green biosynthesis offers an alternative approach which can substantially overcome the environmental challenges posed by physical and chemical methods and do not involve hazardous chemicals and intolerant reaction conditions. The method is based on the ability of environmental microorganisms to remediate metals through enzymatic reduction of metal ions [1]. During this process, metal ions can be converted into nanoparticles of varying sizes and morphologies [2].

Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared spectra of AgNPs were recorded to provide an evidence for the interaction of functional groups involved in the reduction of AgNO$_3$. The NP solutions were dried at room temperature and the powder form was analyzed at a spectral range 4000–400 cm^{-1} at a resolution of 4 cm^{-1} (Thermo Scientific Nicolet iS10 FT-IR spectrometer). The FT-IR analysis of cell-free extracts was also performed which served as a reference.

Stability of AgNPs

The synthesized AgNP solutions were stored at 30 °C under normal light conditions. The stability of green silver NPs under electrolytic conditions in the presence of sodium chloride (NaCl) were studied by adding aliquots of 5 M NaCl into 2 mL of AgNP solution. UV–Visible spectra were obtained in the range of 350–800 nm. Variability of the UV–visible absorbance with NaCl concentration was compared. The procedure was repeated for chemogenized AgNP solution synthesized according to Rashid et al. [15] for comparison.

Antimicrobial Activity of Nanoparticles

Agar Diffusion Assay

The antimicrobial activity of synthesized AgNPs against *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, Methicillin resistant *S. aureus* (MRSA) (clinical strain) and *Candida albicans* ATCC 10231 were determined using the well diffusion assay. Organisms were cultured overnight at 37 °C and suspensions of 0.5 McFarland standard were prepared for each organism. Standard inocula (100 µL) of each bacterium or *Candida* was lawned on Mueller–Hinton Agar (MHA, Himedia, India)/Sabourauds Dextrose Agar (SDA, Himedia, India). Wells were cut in the agar medium using a sterile 9 mm cork borer. The bottom of the wells were sealed with molten agar. Wells were loaded with green AgNP solution, negative controls (consisting of culture supernatants of each organism and sterile nutrient broth) and positive control (0.5% AgNO$_3$ solution) and incubated at 37 °C for 24 h. All experiments were done in triplicates. Zones of inhibition (ZOI) were measured and the average of ZOI was calculated.

Plate Coating Method

Sterile 6 cm petri dishes were coated with (A) Biosynthesized silver NPs and (B) bacterial culture supernatant as the negative control. The prepared nanoparticle solutions (2 mL) were added into individual petri dishes and allowed to completely dry for 24 h inside a class II biological safety cabinet. Subsequently 2 mL of bacterial suspension (0.5 McFarland standard) was added to each petri dish and incubated for different time intervals (5, 10, 15, 30, 45, 60 min) at room temperature. Viable cell counts at different contact time points were obtained by spread plate method. The number of colony forming units (CFU) per millilitre was calculated at each incubation time. All experiments were carried out in triplicates.

Statistical Analysis

The viable counts obtained at different contact times were used to calculate the percentage reduction using the following equation.

$$Average\ percentage\ reduction = \frac{CFU/\text{ml } in\ silver\ NPs}{CFU/\text{ml } in\ control} \times 100$$

For graph preparation, OriginPro 9.0 software was used.

Results and Discussion

Optimization of Culture Conditions for AgNP Synthesis

In this study, all the selected organisms synthesized AgNPs successfully under different optimized conditions. AgNP biosynthesis can be affected by multiple factors such as the culture conditions including pH, temperature, culture medium and the microbial species [16]. Microbial reduction of Ag ions into NPs can be carried out intracellularly or extracellularly [2, 8]. Culture supernatants contain microbial products and media components that facilitate this reaction extracellularly [8]. Proteins such as nitrate
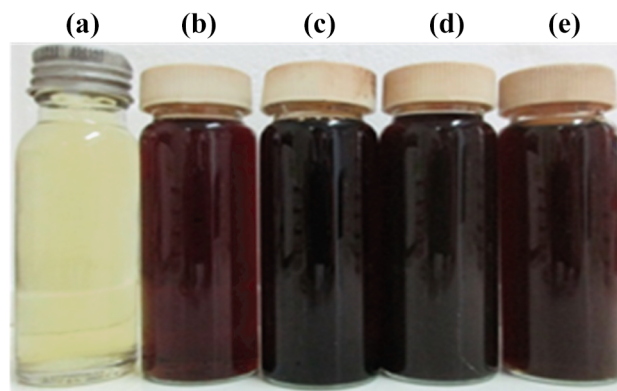
Fig. 1 The cell free filtrates, **a** without AgNO$_3$ and after addition of AgNO$_3$ for biosynthesis of AgNPs, **b** *P. aeruginosa*, **c** *E. coli*, **d** *A. baumannii* and **e** *S. aureus* with AgNO$_3$ after 72 h of incubation

Table 1 Optimized culture conditions for AgNP biosynthesis

Organism	Optimized culture conditions			
	AgNO$_3$ concentration (g/L)	Culture pH	Incubation temperature (°C)	Incubation time (h)
P. aeruginosa (P-AgNPs)	0.4	9.0	70	96
E. coli (E-AgNPs)	0.4	9.0	60	72
A. baumannii (A-AgNPs)	0.4	9.0	60	72
S. aureus (S-AgNPs)	0.4	9.0	60	72

reductase stabilize NPs and reduce aggregation of particles which help to maintain the nano size [1].

Addition of AgNO$_3$ to the four culture supernatants resulted in a colour change from pale yellow to dark brown indicating AgNP synthesis within one hour (Fig. 1). The intensity of the colour of the solution increased further during incubation. The UV–Visible spectra obtained around 420–435 nm range confirmed AgNP formation in this study. The optimum AgNO$_3$ concentrations for all four supernatants were 0.4 g/L (Table 1). Other studies have reported varying time parameters for the occurrence of colour change resulting in AgNP biosynthesis, [17, 18]. The observed color development is also known to be associated with the shape and size of AgNPs [19, 20].

Alkaline pH favoured optimum AgNP biosynthesis. Optimum pH for the biosynthesis of all four AgNP samples was pH 9. The optimum incubation temperatures were 70 °C for P-AgNPs, 60 °C for E-AgNPs, A-AgNPs and S-AgNPs while maximum UV absorbance was observed at 72 h for three of the AgNPs except for P-AgNPs where the optimum incubation time was 96 h (Table 1). Van Dong et al. [19] described the effect of pH on the size and shape of the synthesized AgNPs where at high alkaline pH, spherical and rod shaped AgNPs were produced due to the fast reaction rate while triangular or polygonal shaped
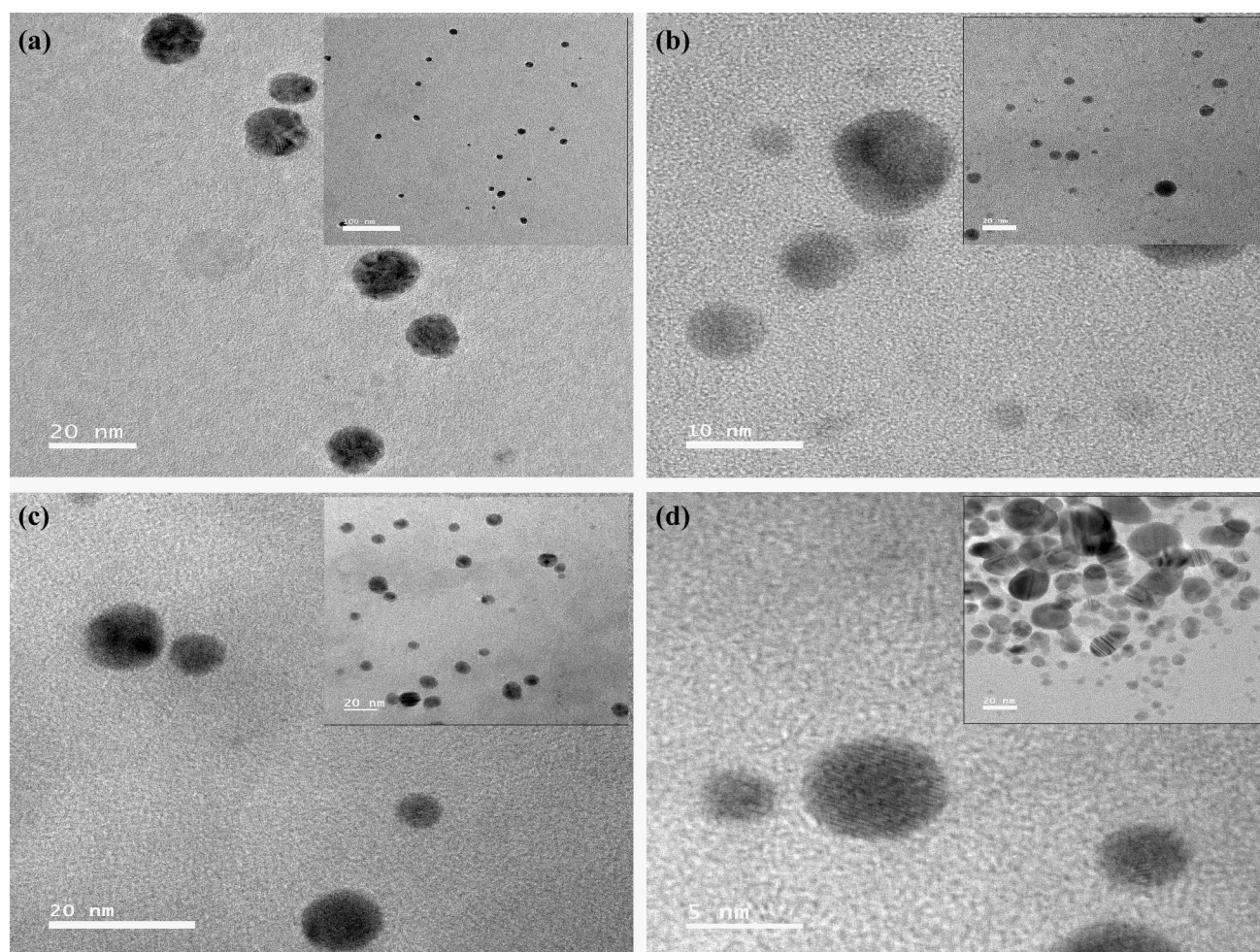
Fig. 2 TEM images of AgNPs synthesized from different bacteria. **a** P-AgNPs, **b** E-AgNPs, **c** A-AgNPs and **d** S-AgNPs (scale bar given)

Fig. 3 Particle size distribution of AgNPs synthesized from different bacteria. **a** P-AgNPs, **b** E-AgNPs, **c** A-AgNPs and **d** S-AgNPs
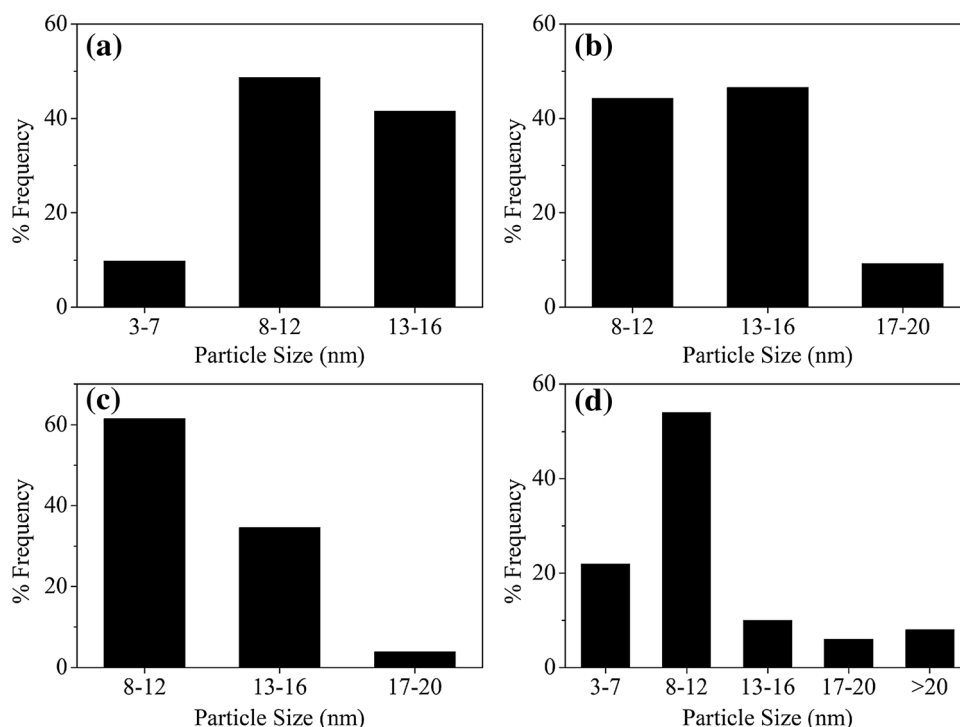
AgNPs resulted at low pH due to a slow reaction rate. AgNPs have several reported morphologies including triangular [21], polyhedral [21, 22], nanoprisms [19], spherical [19, 21] and rod shape [21]. These results indicate the impact of the culture conditions on the microbial metabolic activities which can be harnessed for efficient AgNP production.

The AgNO$_3$ concentration can also affect the size and morphology of the AgNPs. At higher AgNO$_3$ concentrations there is an increase in the rate of reduction of silver ions to AgNPs [14]. High silver salt concentrations can result in aggregated NPs with larger size [23]. In a study by Singh et al. [10] 0.7 mM AgNO$_3$ concentration was found to be optimum for AgNP synthesis by *Acinetobacter calcoaceticus*. The present study reports AgNP biosynthesis by a clinical isolate of *A. baumannii*. To our knowledge, Silver nanoparticle biosynthesis by *A. baumannii* has not been reported previously. The microbial AgNP synthesis was favoured by alkaline pH while under acidic conditions, particles tend to aggregate and become unstable [24].

Morphology and Size Determination of AgNPs by Transmission Electron Microscopy

The morphology of AgNPs biosynthesized at the optimized parameters were determined using TEM. In this study, all AgNPs were found to be of spherical morphology (Fig. 2). The average sizes of the NPs were 11.14 ± 6.59 nm (S-AgNPs), 11.71 ± 2.73 nm (P-AgNPs), 12.87 ± 2.95 nm

(E-AgNPs) and 12.22 ± 2.45 nm (A-AgNPs). S-AgNPs had the largest size distribution having a size range between 3.4–33.0 nm. An important finding was that all other bacterial synthesized NPs resulted in less than 20 nm size AgNPs (Fig. 3).

When considering the TEM results the smaller AgNPs were produced by *S. aureus* and *P. aeruginosa* which gave a UV–visible absorbance peak at 430 and 427 nm respectively. The highest frequency of AgNPs by *S. aureus*, *P. aeruginosa* and *A. baumannii* were of the size range 8–12 nm while for *E. coli* it was 13–16 nm. According to other studies [1, 25] the optimized conditions in this study resulted in very small AgNPs than previously reported for *P. aeruginosa*, *S. aureus* (< 8 nm) indicating stronger antimicrobial potential due to high surface area to volume ratio. Table 2 shows a comparison of AgNPs from various bacterial sources found in recent literature.

FT-IR Analysis

FT-IR analysis of P-AgNPs revealed four prominent bands at 1643, 1586, 1397 and 1042 cm^{-1}. A-AgNPs had FT-IR bands at 1643.75, 2161.48 and 2924.28 cm^{-1} while, E-AgNPs had one prominent band at 2916.88 cm^{-1}. FT-IR of S-AgNPs, demonstrated bands at 2164.86, 2049.17 and 1979.69 cm^{-1} (Fig. 4).

The biosynthesized silver nanoparticles have increased stability due to the coating of the NPs by microbial and media components [26]. In the FTIR spectra (Fig. 4), 2924,

Table 2 Biological synthesis of AgNPs by various microorganisms

Microorganism	Particle size (nm)	Particle structure and distribution	Maximum absorption wavelength (nm)	References
Bacillus cereus	2–16	Spherical	420	[9]
P. aeruginosa strain BS-161R	8–24	Spherical, anisotropic	430	[26]
P. aeruginosa	20–50	Spherical, monodispersed	455	[1]
P. aeruginosa strain KUPSB12	50–85	Spherical	442	[27]
Aspergillus flavus strain NJP08	10–35	Spherical, monodispersed	421	[11]
Acinetobacter calcoaceticus strain LRVP54	8–12	Crystalline, polydispersed	410–440	[10]
E. coli	20–50	Spherical	400	[28]
Penicillium strain	> 100	Colloidal, polydispersed	440	[24]
P. aeruginosa ATCC 27853	33–300	Spherical	420–430	[14]
Bacillus sp	65–70	Spherical and pseudospherical	420	[29]
A. flavus strain NCIM650	37.3–52.92	nd	420	[30]
Emericella nidulans strain APF4	10–450	Hexagonal, monodispersed	450	[31]
P. aeruginosa	20–100	Crystalline	420–430	[18]
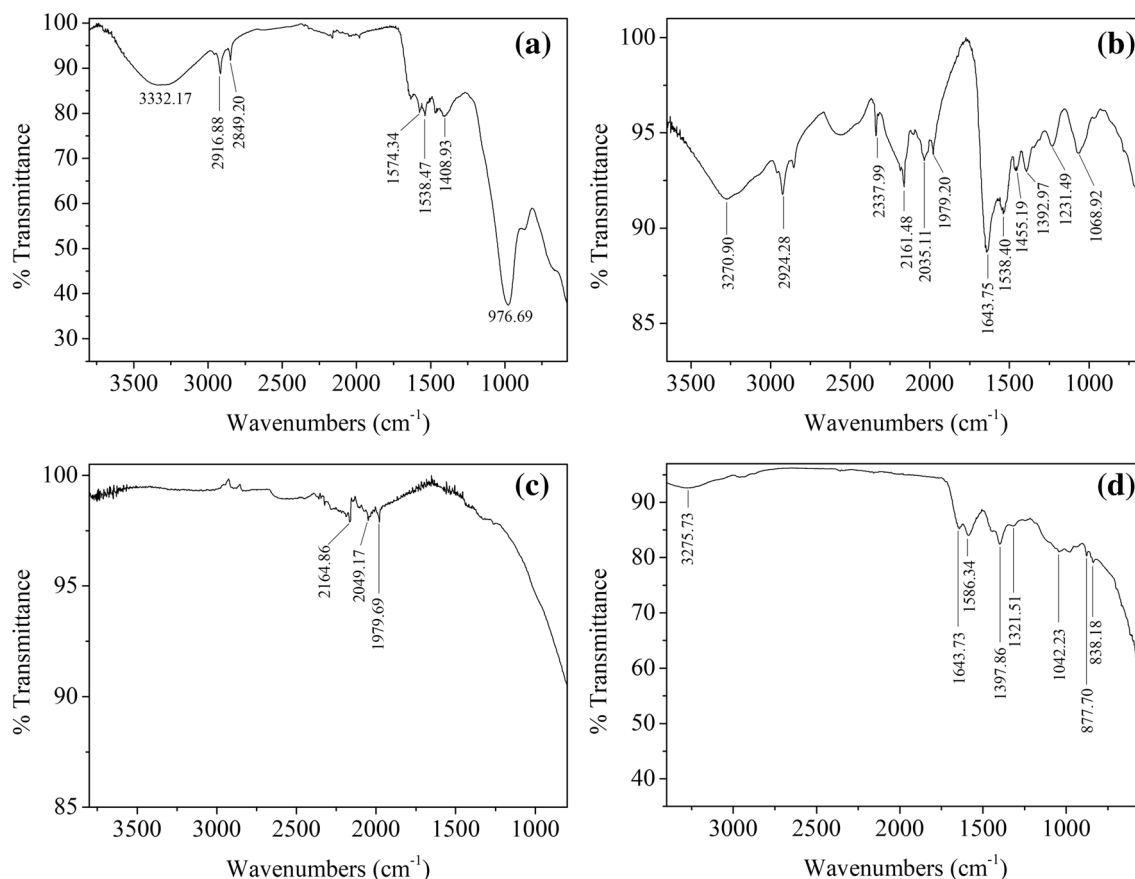
Fig. 4 FT-IR spectra of AgNPs. **a** E-AgNPs, **b** A-AgNPs, **c** S-AgNPs and **d** P-AgNPs
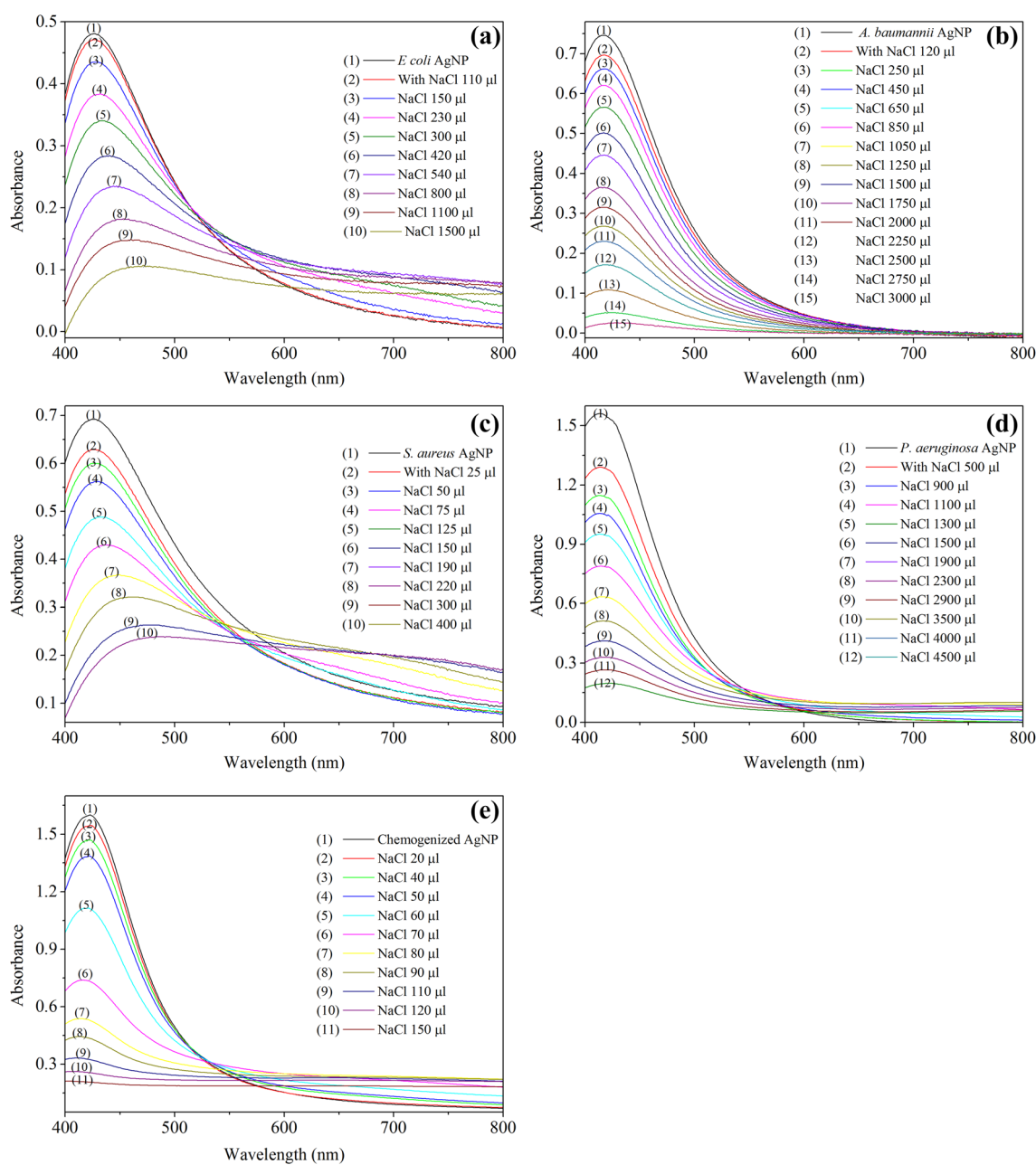
Fig. 5 Stability of **a** E-AgNPs, **b** A-AgNPs, **c** S-AgNPs, **d** P-AgNPs and **e** Chemogenized AgNPs in the presence of NaCl

Table 3 Average zones of inhibition of AgNPs from four different bacteria against selected pathogens

Test organism	Average ZOI (mm) obtained from various bacterial AgNPs				
	E. coli	*A. baumannii*	*S. aureus*	*P. aeruginosa*	0.5% AgNO$_3$ solution
E. coli	10.0	13.3	14.7	13.0	16.0
P. aeruginosa	10.0	14.7	13.0	12.0	17.7
S. aureus	–	–	12.7	12.3	12.0
MRSA	–	–	12.7	12.7	12.0
C. albicans	–	10.0	12.7	14.0	10.0
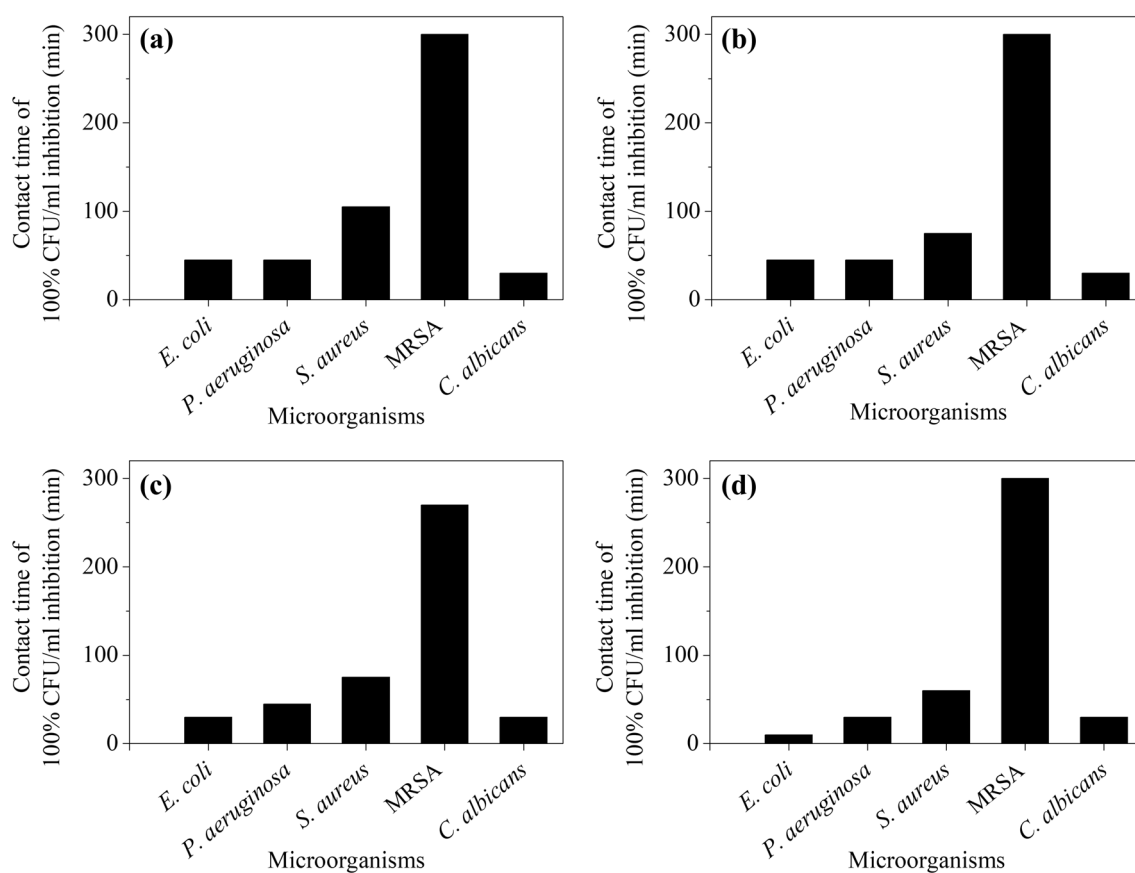
Fig. 6 Contact time required for 100% inhibition of selected pathogens by AgNPs. **a** E-AgNPs, **b** A-AgNPs, **c** S-AgNPs and **d** P-AgNPs in CFU/mL

2916, 1538 and 1455 cm^{-1} modes are due to NH stretch vibrations present in the amide linkages of primary and secondary amines of proteins [32–34]. The peak 1397 cm^{-1} shows the presence of nitrocompounds from proteins or enzymes [35]. The peak at 2849 cm^{-1} can be assigned to the C–H symmetrical stretch vibration of alkanes [32]. According to Jeevan et al. [18] a band around 1643 cm^{-1} in P-AgNP and A-AgNP samples, may indicate –C=O carbonyl groups and –C=C– stretching. Percentage transmittance of 1042 cm^{-1} refers to –C–N– stretching vibrations. This shows the presence of proteins in the biologically synthesized AgNP solutions which support stabilization of AgNPs. Protein components of the medium can bind to AgNPs through free amino or cysteine groups and cause capping of biosynthesized AgNPs which can help to stabilize the nanoparticles in solution and prevent agglomeration [36].

Stability of Green AgNPs

The stability of the biosynthesized AgNPs and the chemogenized AgNPs were compared in the presence of electrolytes (5 M NaCl) (Fig. 5).

The stability of AgNPs is considered to be inversly propotional to the rate of flocculation. The P-AgNPs and A-AgNPs remained stable even after adding more than 3000 μL of 5 M NaCl, however S-AgNPs lost their stability at much lower volume (150 μL) of 5 M NaCl while E-AgNPs were unstable after addition of 1500 μL of 5 M NaCl. Of the four NPs, P-AgNPs were the most stable followed by A-AgNPs and E-AgNPs while S-AgNPs were found to be least stable. Chemogenized AgNPs were found to be less stable compared to biosynthesized AgNPs suggesting that membrane bound proteins prevent aggregation of green AgNPs thereby promoting stability [37].

Antimicrobial Activity

Zones of inhibition (ZOI) were observed for AgNPs synthesized by all organisms tested. E-AgNPs exhibited inhibition zones against *E. coli* and *P. aeruginosa*, while *S. auerus*, MRSA and *C. albicans* did not show any ZOI. A-AgNPs had ZOI against *E. coli, P. aeruginosa* and *C. albicans* (Table 3).

In this study, well diffusion results and plate coating results suggest that E-AgNPs had the lowest antimicrobial

activity while P-AgNPs and S-AgNPs had highest antimicrobial activity. In the well diffusion study, only P-AgNPs and S-AgNPs resulted in inhibition of Gram positive test organisms *S. aureus* and MRSA.

The plate coating method was performed to determine the percentage inhibition of CFU/mL at different contact times in the presence of the tested AgNPs. The results of the plate coating assay indicates that AgNPs synthesized by all four microorganisms could completely (100%) inhibit *P. aeruginosa*, *E. coli* and *C. albicans* when in contact for less than 60 min. P-AgNPs, A-AgNPs and S-AgNPs completely inhibited *S. aureus* after 75 min, while E-AgNPs completely inhibited *S. aureus* following 105 min of contact. However, complete inhibition of MRSA was achieved after 300 min of contact with P-AgNPs, E-AgNPs and A-AgNPs while for S-AgNPs, the contact time required was 270 min (Fig. 6).

Antimicrobial properties of synthesized AgNPs were higher against gram negative bacteria compared to Gram positive bacteria possibly due to the difference in cell membrane thickness [9, 28, 38]. Spherical silver NPs have increased antimicrobial activity due to high surface area to volume ratio [10]. Their larger surface area favours binding with various ligands. According to Cicek et al. [39] due to its smaller size, AgNPs can move into microbial cells through cell membranes and the antimicrobial activity tends to increase with the reduced size. Silver NPs can get attached to the cell membranes and release silver ions slowly [1]. AgNPs is suggested to cause pit formation in the cell wall of bacteria resulting in increased permeability and leakage of cellular contents through the plasma membrane. Further a study by Song et al. [40] reported a higher concentration of AgNPs were required against Gram positive bacteria compared to Gram negative bacteria. This observation which supports our findings can be explained by the difference in the bacterial cell wall, where Gram positive bacteria contain a thick cell wall containing peptidoglycan which can resist the passage of the nanoparticles compared to the thinner cell wall of Gram negative bacteria. Evidence shows release of Ag ions from AgNPs within the cell and on the cell membrane [41], where the silver ions are suggested to interact with thiol groups on proteins thereby altering the structure and functions of enzymes resulting in bacterial death [42]. Further, the negatively charged Gram positive bacterial cell wall bind with more Ag ions which results in less number of AgNPs interacting with the plasma membrane thereby protecting the plasma membrane from the reactive oxygen species generated by the AgNPs leading to increased resistance observed. [43]. Further silver NPs mediate binding with DNA, protein denaturation, and respiratory functions leading to cell death [1, 9, 38]. In conclusion, AgNPs synthesized from selected bacteria yielded less than 20 nm

NPs which showed effective antimicrobial activity and was found to be a cost effective and eco-friendly method.

References

1. Oza G, Pandey S, Shah R, Sharon M (2012) Extracellular fabrication of silver nanoparticles using *Pseudomonas aeruginosa* and its antimicrobial assay. Pelagia Res Lib Adv Appl Sci Res 3:1776–1783. https://www.researchgate.net/profile/Dr_Sunil_Pandey2/publication/230707314_Extracellular_Fabrication of Silver Nanoparticles using *Pseudomonas aeruginosa* and its Antimicrobial Assay/links/0912f5034 8c53f0b10000000.pdf. Accessed 27 Aug 2017

2. Li X, Xu H, Chen Z-S, Chen G (2011) Biosynthesis of nanoparticles by microorganisms and their applications. J Nanomater 2011:1–16. https://doi.org/10.1155/2011/270974

3. Otari SV, Patel SK, Jeong J-H, Lee JH, Lee J-K (2016) A green chemistry approach for synthesizing thermostable antimicrobial peptide-coated gold nanoparticles immobilized in an alginate biohydrogel. RSC Adv 6:86808–86816. https://doi.org/10.1039/C6RA14688K

4. Hölken I, Hoppe M, Mishra YK (2016) Complex shaped ZnO nano-and microstructure based polymer composites: mechanically stable and environmentally friendly coatings for potential antifouling applications. Phys Chem Chem Phys 18:7114–7123. https://doi.org/10.1039/C5CP07451G

5. Hunagund SM, Desai VR, Barretto DA, Pujar MS, Kadadevarmath JS, Vootla S, Sidarai AH (2017) Photocatalysis effect of a novel green synthesis gadolinium doped titanium dioxide nanoparticles on their biological activities. J Photochem Photobiol 346:159–167. https://doi.org/10.1016/j.jphotochem.2017.06.003

6. Patel SK, Lee J-K, Kalia VC (2017) Nanoparticles in biological hydrogen production: an overview. Indian J Microbiol. https://doi.org/10.1007/s12088-017-0678-9

7. Otari S, Pawar S, Patel SK, Singh RK, Kim S-Y, Lee JH, Zhang L, Lee J-K (2017) *Canna edulis* leaf extract-mediated preparation of stabilized silver nanoparticles: characterization, antimicrobial activity, and toxicity studies. J Microbiol Biotechnol 27:731–738. https://doi.org/10.4014/jmb.1610.10019

8. Singh R, Shedbalkar UU, Wadhwani SA, Chopade BA (2015) Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. Appl Microbiol Biotechnol 99:4579–4593. https://doi.org/10.1007/s00253-015-6622-1

9. Gurunathan S (2014) Rapid biological synthesis of silver nanoparticles and their enhanced antibacterial effects against *Escherichia fergusonii* and *Streptococcus mutans*. Arab J Chem (**in press**)

10. Singh R, Wagh P, Bellare J (2013) Synthesis, optimization, and characterization of silver nanoparticles from *Acinetobacter calcoaceticus* and their enhanced antibacterial activity when combined with antibiotics. Int J Nanomed 8:4277–4290. https://doi.org/10.2147/IJN.S48913

11. Jain N, Bhargava A, Majumdar S, Tarafdar J, Panwar J (2011) Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* njp08: a mechanism perspective. Nanoscale 3:635–641. https://doi.org/10.1039/c0nr00656d

12. Krishna IM, Reddy GB, Veerabhadram G, Madhusudhan A (2016) Eco-friendly green synthesis of silver nanoparticles using *Salmalia malabarica*: synthesis, characterization, antimicrobial, and catalytic activity studies. Appl Nano Sci 6:681–689. https://doi.org/10.1007/s13204-015-0479-6

13. Park TJ, Lee SY, Heo NS, Seo TS (2010) In vivo synthesis of diverse metal nanoparticles by recombinant *Escherichia coli*. Angew Chem Int Ed 49:7019–7024. https://doi.org/10.1002/anie.201001524

14. Peiris MK, Gunasekara CP, Jayaweera PM, Arachchi ND, Fernando N (2017) Biosynthesized silver nanoparticles: are they effective antimicrobials? Mem Inst Oswaldo Cruz 112:537–543. https://doi.org/10.1590/0074-02760170023

15. Rashid MU, Bhuiyan MKH, Quayum ME (2013) Synthesis of silver nano particles (Ag-NPs) and their uses for quantitative analysis of vitamin c tablets. Dhaka Univ J Pharm Sci 12:29–33. https://doi.org/10.3329/dujps.v12i1.16297

16. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SRK, Muniyandi J, Hariharan N, Eom SH (2009) Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Surf B 74:328–335. https://doi.org/10.1016/j.colsurfb.2009.07.048

17. El-Shanshoury AE-RR, ElSilk SE, Ebeid ME (2011) Extracellular biosynthesis of silver nanoparticles using *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Streptococcus thermophilus* ESh1 and their antimicrobial activities. ISRN Nanotechnol 2011:1–7. https://doi.org/10.5402/2011/385480

18. Jeevan P, Ramya K, Rena AE (2012) Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*. Indian J biotechnol 11:72–76 https://pdfs.semanticscholar.org/885a/b93862e3deb07611b4325c3234a7890e3b6d.pdf. Accessed 15 June 2016

19. Van Dong P, Ha CH, Binh LT, Kasbohm J (2012) Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. Int Nano Lett 2:1–9. https://doi.org/10.1186/2228-5326-2-9

20. Zielińska A, Skwarek E, Zaleska A, Gazda M, Hupka J (2009) Preparation of silver nanoparticles with controlled particle size. Procedia Chem 1:1560–1566. https://doi.org/10.1016/j.proche.2009.11.004

21. Rout A, Jena PK, Sahoo D, Bindhani BK (2014) Green synthesis of silver nanoparticles of different shapes and its antibacterial activity against *Escherichia coli*. Int J Curr Microbiol App Sci 3:374–383 https://www.ijcmas.com/vol-3-4/Anandini%20Rout,%20et%20al.pdf. Accessed 15 Aug 2017

22. Logaranjan K, Raiza AJ, Gopinath SCB, Chen Y, Pandian K (2016) Shape- and size-controlled synthesis of silver nanoparticles using *Aloe vera* plant extract and their antimicrobial activity. Nanoscale Res Lett 11:1–9. https://doi.org/10.1186/s11671-016-1725-x

23. Sobczak-Kupiec A, Malina D, Wzorek Z, Zimowska M (2011) Influence of silver nitrate concentration on the properties of silver nanoparticles. IET Micro Nano Lett 6:656–660. https://doi.org/10.1049/mnl.2011.0152

24. Sadowski Z, Maliszewska I, Grochowalska B, Polowczyk I, Kozlecki T (2008) Synthesis of silver nanoparticles using microorganisms. Mater Sci Poland 26:419–424 https://pdfs.semanticscholar.org/b2bc/87b05b455a7d00220465c6d231087e3fbfe4.pdf. Accessed 27 Aug 2017

25. Nanda A, Saravanan M (2009) Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. Nanomedicine 5:452–456. https://doi.org/10.1016/j.nano.2009.01.012

26. Kumar CG, Mamidyala SK (2011) Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*. Colloids Surf B 84:462–466. https://doi.org/10.1016/j.colsurfb.2011.01.042

27. Paul D, Sinha SN (2014) Extracellular synthesis of silver nanoparticles using *Pseudomonas aeruginosa* KUPSB12 and its antibacterial activity. Jordan J of Biol Sci **7**:245–250 http://jjbs.hu.edu.jo/files/v7n4/Paper%20Number%202m.pdf. Accessed 01 Sept 2017

28. Kushwaha A, Singh VK, Bhartariya J, Singh P, Yasmeen K (2015) Isolation and identification of *E. coli* bacteria for the synthesis of silver nanoparticles: characterization of the particles and study of antibacterial activity. Eur J Exp Biol 5:65–70 http://www.imedpub.com/articles/isolation-and-identification-of-iecoliibacteria-for-the-synthesis-of-silver-nanoparticles-characterization-of-the-particles-and.pdf. Accessed 15 Sept 2017

29. Malarkodi C, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Vanaja M, Annadurai G (2013) Bacterial synthesis of silver nanoparticles by using optimized biomass growth of *Bacillus* sp. Nanosci Nanotechnol 3:26–32 https://www.researchgate.net/profile/Rajeshkumar_Shanmugam/publication/236671464_Bacterial_synthesis_of_silver_nanoparticles_by_using_optimized_biomass_growth_of_Bacillus_sp/links/00b7d518d35968b7c9000000.pdf. Accessed 09 June 2017

30. Patil H, Borse S, Patil D, Patil U, Patil H (2011) Synthesis of silver nanoparticles by microbial method and their characterization. Arch Phys Res 2:153–158 https://www.researchgate.net/profile/Ulhas_Patil4/publication/268384142_Synthesis_of_Silver_Nanoparticles_by_Microbial_Method_and_Their_Characterization/links/54e337230cf2de71a71e58a0.pdf. Accessed 09 June 2017

31. Barapatre A, Aadil KR, Jha H (2016) Synergistic antibacterial and antibiofilm activity of silver nanoparticles biosynthesized by lignin-degrading fungus. Bioresour Bioprocess 3:8. https://doi.org/10.1186/s40643-016-0083-y

32. Phanjom P, Ahmed G (2015) Biosynthesis of silver nanoparticles by *Aspergillus oryzae* (MTCC no. 1846) and its characterizations. J Nanosci Nanotechnol 5:14–21. https://doi.org/10.5923/j.nn.20150501.03

33. Devika R, Elumalai S, Manikandan E, Eswaramoorthy D (2012) Biosynthesis of silver nanoparticles using the fungus *Pleurotus ostreatus* and their antibacterial activity. Sci Rep 1:1–5. https://doi.org/10.4172/scientificreports.557

34. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R (2010) Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine 6:103–109. https://doi.org/10.1016/j.nano.2009.04.006

35. Rajeshkumar S, Malarkodi C (2014) In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. Bioinorg Chem Appl 2014:1–10. https://doi.org/10.1155/2014/581890

36. AbdelRahim K, Mahmoud SY, Ali AM, Almaary KS, Mustafa AE-ZM, Husseiny SM (2017) Extracellular biosynthesis of silver nanoparticles using *Rhizopus stolonifer*. Saudi J Biol Sci 24:208–216. https://doi.org/10.1016/j.sjbs.2016.02.025

37. Pandey S, Thakur M, Shah R, Oza G, Mewada A, Sharon M (2013) A comparative study of economical separation and aggregation properties of biologically capped and thiol functionalized gold nanoparticles: selecting the eco-friendly trojan horses for biological applications. Colloids Surf B 109:25–31. https://doi.org/10.1016/j.colsurfb.2013.03.011

38. Okafor F, Janen A, Kukhtareva T, Edwards V, Curley M (2013) Green synthesis of silver nanoparticles, their characterization, application and antibacterial activity. Int J Environ Res Publ health 10:5221–5238. https://doi.org/10.3390/ijerph10105221

39. Cicek S, Gungor AA, Adiguzel A, Nadaroglu H (2015) Biochemical evaluation and green synthesis of nano silver using

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peroxidase from Euphorbia (*Euphorbia amygdaloides*) and its antibacterial activity. J Chem 2015:1–7. https://doi.org/10.1155/2015/486948 **(Hindawi online)**

40. Song H, Ko K, Oh I, Lee B (2006) Fabrication of silver nanoparticles and their antimicrobial mechanisms. Eur Cells Mater 11:58 http://thesilveredge.com/pdf/Fabrication%20of%Silver%20Nanoparticles%20and%20Their%20Antimicrobial%20Mechanisms.pdf. Accessed 15 September 2017

41. Long Y-M, Hu L-G, Yan X-T, Zhao X-C, Zhou Q-F, Cai Y, Jiang G-B (2017) Surface ligand controls silver ion release of nanosilver and its antibacterial activity against *Escherichia coli*. Int J Nanomedicine 12:3193. https://doi.org/10.2147/IJN.S132327

42. AshaRani P, Low Kah Mun G, Hande MP, Valiyaveettil S (2008) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3:279–290. https://doi.org/10.1021/nn800596w

43. Liao J, Mo AC, Wu HK, Zhang JC, Li YB, Lv GY (2007) Antibacterial activity of silver-hydroxyapatite/titania nanoparticles on oral bacteria. Key Eng Mater 330–332:299–302. https://doi.org/10.4028/www.scientific.net/KEM.330-332.299